



FIGURE 1A

Nucleotide sequence of NS2/3 (810-1206) st (SEQ ID. No. 1)

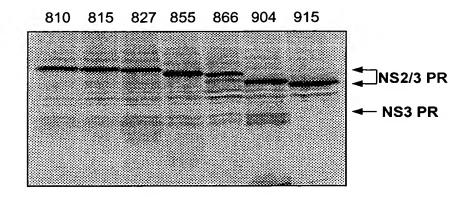
atggaccggg agatggctgc atcgtgcgga ggcgcggttt tcataggtct tgcactcttg accttgtcac catactataa agtgctcctc gctaggctca tatggtggtt acagtattta atcaccagag tegaggegea ettgeaagtg tggateece eteteaatgt teggggagge cgcgatgcca tcatcctcct cacgtgcgca gtccacccag agctaatctt tgacatcacc ttcgtgcgtg cgcaggggct cattcgtgcg tgtatgttgg tgcggaaggc tgcggggggt cattatgtcc aaatggcctt catgaagcta gctgcgctga caggtacgta cgtttatgac catctcactc cattgcagga ttgggcccac gcgggcctac gagaccttgc agtggcggta gaqcccgtca tcttctctga catggaggtc aagatcatca cctggggggc ggacaccgcg gcatgcgggg acatcatttc aggtctgccc gtctccgctc gaaggggaag ggagatactc ctgggaccgg ccgataattt tgaagggcag gggtggcgac tccttgcgcc catcacggcc tactcccaac agacacgggg cctacttggt tgcatcatca ccagcctcac aggccgggac aagaaccagg tcgaggggga ggttcaagtg gtctccaccg ctacacaatc tttcctggcg acctgcgtca acggcgtgtg ttggactgtc ttccatggcg ccggctcaaa gaccttggcc ggccccaaag gcccaatcac ccagatgtac actaatgtgg accaggacct cgtcggctgg caggegeece etggggegeg etceatgaca ceatgeacet geggeagete ggacetetat ttqqtcacqa gacatgccga cgtcattccg gtgcgccggc ggggcgacag tagggggagc ctqctctccc ccaqqcctqt ctcctacttg aagggctctt cgggtggccc actgctctgc cetteqqqqe acqttqtqqq catettecqq getgetgtgt geacecqqqq ggttgcaaaa gcggtggact tcatacctgt tgagtctatg gaaactacca tgcggactag tagcgcttgg cgtcacccgc agttcggtgg t

FIGURE 1B

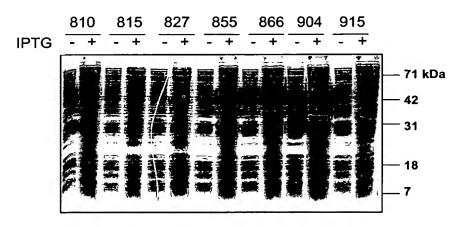
AMINO ACID SEQUENCE OF NS2/3 (810-1206)-ST (SEQ ID NO. 2)

MDREMAASCG GAVFIGLALL TLSPYYKVLL ARLIWWLQYL ITRVEAHLQV WIPPLNVRGG RDAILLTCA
VHPELIFDIT KLLLAIFGPL MVLQAGITKV PYFVRAQGLI RACMLVRKAA GGHYVQMAFM KLAALTGTYV
YDHLTPLQDW AHAGLRDLAV AVEPYIFSDM EVKIITWGAD TAACGDIISG LPVSARRGRE ILLGPADNFE
GQGWRLLAPI TAYSQQTRGL LGCIITSLTG RDKNQVEGEV QVVSTATQSF LATCVNGVCW TVFHGAGSKT
LAGPKGPITQ MYTNVDQDLV GWQAPPGARS MTPCTCGSSD LYLVTRHADV IPVRRGDSR GSLLSPRPVS
YLKGSSGGPL LCPSGHAVGI FRAAVCTRGV AKAVDFIPVE SMETTMRTSS AWRHPQFGG

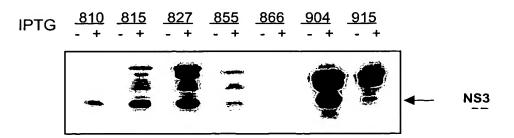
A. in vitro translated N-terminal deletion mutants

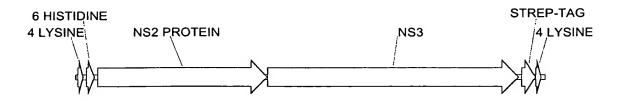


B. E. coli expression - SDS-PAGE

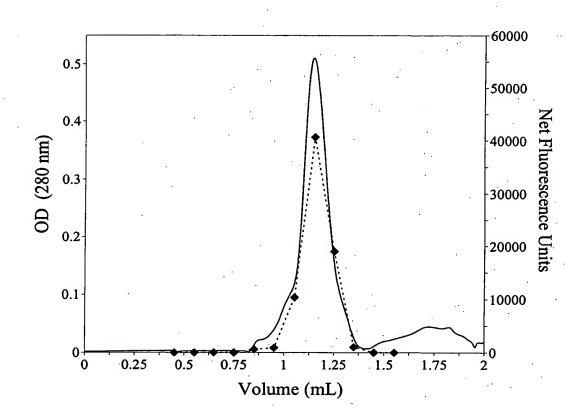


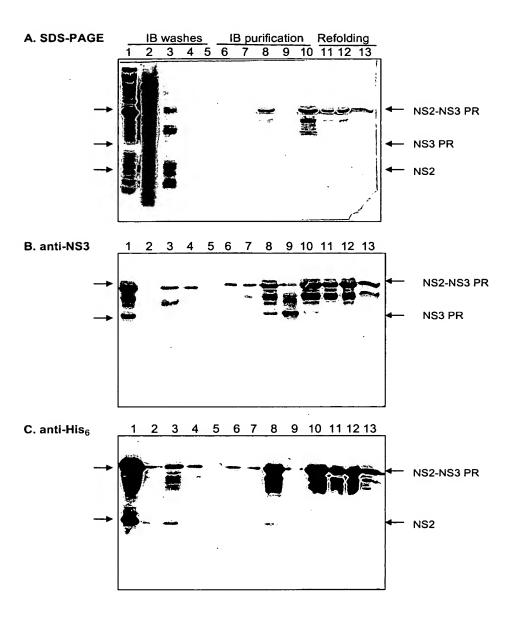
C. E. coli expression - Immunoblot with anti-NS3

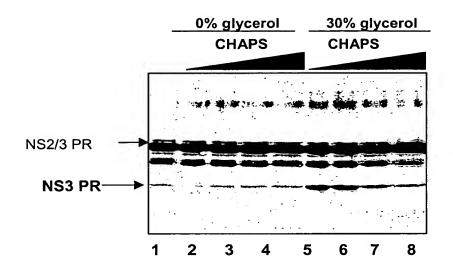


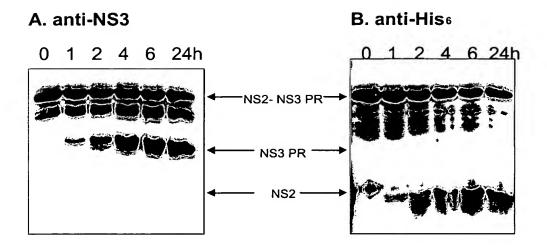


4k 6H NS2/3(904-1206)-ST-4K









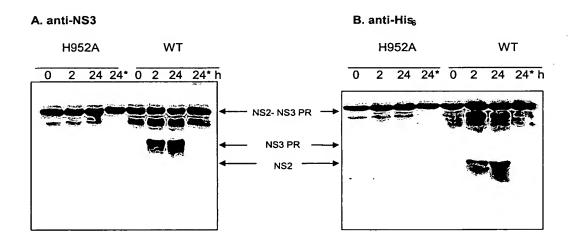


FIGURE 9A

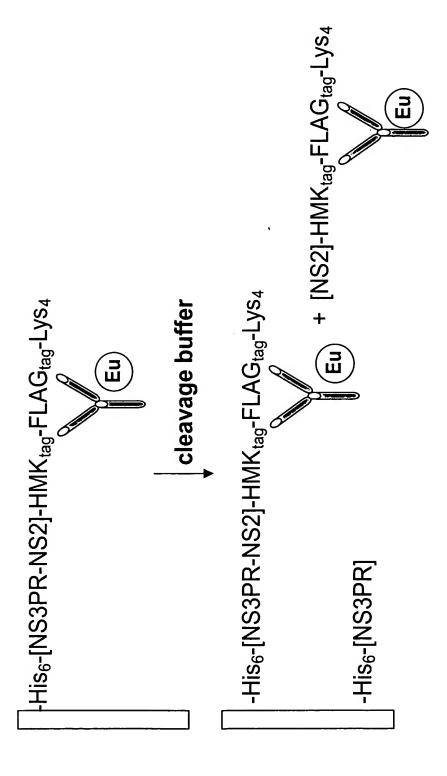
NUCLEOTIDE SEQUENCE OF 4K-6H-NS2/3 (904-1206) ST-4K (SEQ ID NO. 3)

atgaaaaaga	aaaagctcga	gcatcaccat	caccatcaca	ctagtgcagg	cataaccaaa
gtgccgtact	tcgtgcgtgc	gcaggggctc	attcgtgcgt	gtatgttggt	gcggaaggct
gcggggggtc	attatgtcca	aatggccttc	atgaagctag	ctgcgctgac	aggtacgtac
gtttatgacc	atctcactcc	attgcaggat	tgggcccacg	cgggcctacg	agaccttgca
gtggcggtag	agcccgtcat	cttctctgac	atggaggtca	agatcatcac	ctggggggcg
gacaccgcgg	catgcgggga	catcatttca	ggtctgcccg	tctccgctcg	aaggggaagg
gagatactcc	tgggaccggc	cgataatttt	gaagggcagg	ggtggcgact	ccttgcgccc
atcacggcct	actcccaaca	gacacggggc	ctacttggtt	gcatcatcac	cagcctcaca
ggccgggaca	agaaccaggt	cgaggggag	gttcaagtgg	tctccaccgc	tacacaatct
ttcctggcga	cctgcgtcaa	cggcgtgtgt	tggactgtct	tccatggcgc	cggctcaaag
accttggccg	gccccaaagg	cccaatcacc	cagatgtaca	ctaatgtgga	ccaggacctc
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agggggagcc	tgctctcccc	caggcctgtc	tcctacttga	agggctcttc	gggtggccca
ctgctctgcc	cttcggggca	cgttgtgggc	atcttccggg	ctgctgtgtg	cacccggggg
gttgcaaaag	cggtggactt	catacctgtt	gagtctatgg	aaactaccat	gcggactagt
agcgcttggc	gtcacccgca	gttcggtggt	aaaaagaaaa	agtaaggatc	С

FIGURE 9B Amino acid sequence of 4K-6H-NS2/3 (904-1206) ST-4K (SEQ ID NO. 4)

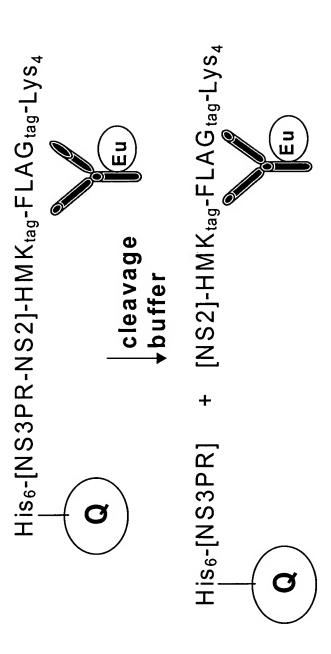
MKKKKLEHHH	${\tt HHHTSAGITK}$	VPYFVRAQGL	IRACMLVRKA	AGGHYVQMAF	MKLAALTGTY	60
VYDHLTPLQD	WAHAGLRDLA	VAVEPVIFSD	MEVKIITWGA	DTAACGDIIS	GLPVSARRGR	120
EILLGPADNF	EGQGWRLLAP	ITAYSQQTRG	LLGCIITSLT	GRDKNQVEGE	VQVVSTATQS	180
FLATCVNGVC	WTVFHGAGSK	TLAGPKGPIT	${\tt QMYTNVDQDL}$	VGWQAPPGAR	SMTPCTCGSS	240
DLYLVTRHAD	VIPVRRRGDS	RGSLLSPRPV	SYLKGSSGGP	LLCPSGHAVG	IFRAAVCTRG	300
VAKAVDFIPV	ESMETTMRTS	SAWRHPOFGG	KKKK			337





8 / 12

FIGURE 11



His₆-[NS3PR-NS2]-HMK_{tag}-FLAG_{tag}-Lys₄

fluorescent probe (potent NS3 protease inhibitor)

cleavage buffer

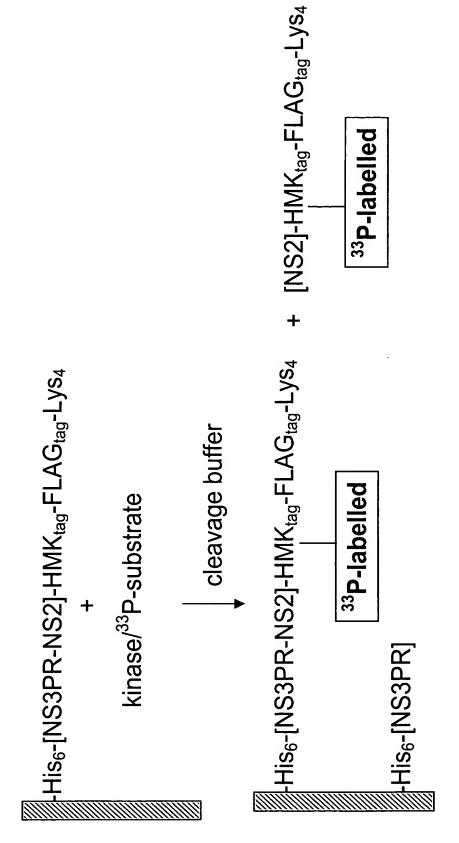
His₆-[NS3PR] His₆-[NS3P_R-NS2]-HMK_{tag}-FLAG_{tag}-Lys₄ +

S₄ + His₆-[NS;

Ľ.

+ [NS2]-HMK_{tag}-FLAG_{tag}-Lys₄





11/12

Strep_{tag}-[NS3PR-NS2]-His₆-Lys₄

50 mM Hepes, pH 7.5, 30% glycerol, 0.5% n-dodecyl-β-D-maltoside, 1 mM TCEP 1.5 h

Strep_{tag}-[NS3PR-NS2]-His₆-Lys₄ + Strep_{tag}-[NS3PR] + [NS2]-His₆-Lys₄



Streptavidin coated plate



Eu⁺³-labeled anti-NS2 (signal decrease assay)

washes

TRF detection